MAIZE GENETICS

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COMPLETION OF THE STUDY OF THE ALLELIC RELATIONS OF DEFICIENCY MUTANTS

During this year studies were completed of 13 mendelizing recessive mutants associated with homozygous small terminal deficiencies of the short arm of chromosome 9. All 13 mutants arose independently following breakage of the short arm of a chromosome 9 which resulted, in each case, in deletion of a small terminal segment. Seven of these mutants gave a pale-yellow seedling phenotype (designated *pyd* 1 to 7) and six gave a white seedling phenotype (designated *wd* 1 to 6). A detailed description of these mutants, their method of origin, and the extent of the deficiencies was given in Year Book No. 42. Completion of these studies consisted in (1) verifying the allelic if not identical nature of all 7 *pyd* mutants, (2) verifying the allelic nature of all 6 *wd* mutants, (3) determining that all 7 *pyd* mutants were allelic to and dominant over all 6 *wd* mutants, (4) determining that all 7 *pyd* mutants were not allelic to the recessive mutant *yg-2* (yellow-green plants), and (5) determining that all 6 *wd* mutants were allelic and recessive to *yg-2*. The results of the completed tests confirm the interpretation of the anomalous allelic relations given in the previous report.

THE CHROMOSOME-BREAKAGE MECHANISM AS A MEANS OF PRODUCING DIRECTED MUTATIONS

The 7 *pyd* and 6 *wd* mutants intensively studied illustrate the repeated occurrence of phenotypically and genetically similar mutants following breakage of the short arm of chromosome 9. If large numbers of newly broken chromosomes 9 could be tested, many more *pyd* and *wd* mutants should appear. The following method was used to test 3287 newly broken chromo-

somes 9 for the presence of the *pyd* or *wd* deficiency mutants among these chromosomes: Large numbers of functional male gametes containing recently broken chromosomes 9 may be obtained by the method outlined in last year's report. Pollen with a large proportion of grains carrying a recently broken chromosome 9 was placed on the silks of plants possessing one normal chromosome 9 and one chromosome 9 with a female-transmissible long terminal deficiency. Whenever male gametes with recently broken chromosomes 9 are delivered by pollen tubes to female gametes, kernels with morphologically normal endosperms will arise when the female gametophyte contributes the normal chromosome 9. In contrast, aberrant endosperms will be formed when the female gametophyte contributes the chromosome 9 with a long terminal deficiency of the short arm. This is because the broken chromosome 9 delivered by the male parent undergoes the chromatid type of breakage-fusion-bridge cycle during the development of the endosperm tissues. This mechanism brings about deletions of terminal segments of the short arm of this chromosome 9 in some cells. Since the chromosomes 9 delivered by the female parent are already deficient for a long terminal segment, the telophase nucleus which receives this newly broken male chromosome 9 will be homozygous deficient for a segment of the short arm of chromosome 9. In such nuclei, the extent of the homozygous deficiency may range from minute to the full extent of the deficiency of the chromosomes 9 delivered by the female parent. Cells having any of these homozygous deficiencies are viable and capable of multiplication. Cells that are homozygous deficient for the longer deficiencies produce sectors within the endosperm that are aberrant either in aleurone color development or in growth rates. These sectors are readily recognizable. It is possible, then, to select from an ear kernels that have received a deficient chromosome 9 from the female parent and a recently broken chromosome 9 from the male parent. In the recently broken chromosome 9 delivered to the zygote, the chromatid type of breakage-fusion-bridge cycle usually ceases in the young sporophytic tissues. The broken end no longer undergoes fusions, and the mitotic behavior of this chromosome is normal from then on. If this healed broken chromosome 9 has at least a full genomic complement of the short arm, green seedlings should arise from the embryos of these kernels. If it has a short terminal deficiency, either pale-yellow or white seedlings could appear. If it has a long terminal deficiency, the embryos homozygous for these longer deficiencies would be expected to be inviable. A total of 3287 seedlings was obtained from kernels that were classified by their endosperm appearance as having received a deficient chromosome 9 from the female parent and a newly broken chromosome 9 from the male parent. Of these seedlings, 77 were typical pale-yellow mutants in phenotype, and 48 were typical white mutants. Although tests for allelism of these 125 new mutants could not be made with the 7 *pyd* and 6 *wd* mutants intensively studied, the methods of origin and detection of these mutants make it difficult to conclude that they do not represent the repeated occurrence of phenotypically and genetically similar mutants. From the intensive study of the 7 *pyd* and 6 *wd* mutants alone, it is obvious that the chromosome-breakage mechanism is a means of independently inducing the same mutations time and again. In this respect, the mutation process is directed.

Continuation of the Chromatid Type of Breakage-Fusion-Bridge Cycle in the Sporophytic Tissues

Among the seedlings which arose from the study outlined in the foregoing section, a type of behavior of the recently broken chromosome 9 delivered by the male parent was observed that had previously not been recognized. All the kernels from one ear that had received a deficient chromosome from the female parent and a recently broken chromosome from the male parent were planted under a single culture number. In many cultures, the green seedlings arising from these kernels were normal in appearance. In some cultures, a few seedlings were variegated for fine streaks of colorless, defective tissue. In a very few cultures, 20 to 30 per cent of the green seedlings were so variegated. All degrees of variegation were represented among these seedlings, some showing only a few small sectors of variegated tissue whereas others were variegated throughout. The kind of variegation strongly suggested that the chromatid type of breakage-fusion-bridge cycle had not ceased in the young embryo, but that the chromosome with the broken end was continuing this process into the sporophytic tissues. If this were true, just such variegation would be expected, because the chromatid type of breakage-fusion-bridge cycle would constantly delete segments from the short arm of the chromosome 9 delivered by the male parent. Since the chromosome 9 delivered by the female parent was already deficient for a relatively long terminal segment, nuclei—and thus sectors of homozygous deficient tissues—could be produced. These tissues should have chlorophyll abnormalities, since it is known that a homozygous deficiency for the tip of the short arm results in absence of chlorophyll. The tissues with the longer homozygous deficiencies could be expected to grow at a slower rate than tissues with a full genomic complement. Thus, the fine streaks with chlorophyll modifications might represent these sectors of homozygous deficient tissues.

In most cases, the relative amount of variegated tissue diminished in the older leaves. Sectors of nonvariegated tissue were constantly arising as the plant developed. In no case was the whole plant variegated at the time of maturity. This suggested that the breakage-fusion-bridge cycle had ceased in the precursor cell or cells of these sectors, and that the broken end of chromosome 9 had healed and was no longer undergoing sister chromatid fusions.

Cytological examination of the anaphase figures in young leaves, in young shoots, and in the growing glumes of the florets, and of the meiotic division figures confirmed the interpretation. In those regions of a plant where it could be concluded that no variegation was occurring, no bridges at anaphase were found. In the parts of the plant where it could be expected that variegation for defective tissue was continuing, a single chromosome producing a bridge configuration at anaphase was observed in many figures. To determine whether a single chromatid bridge occurs in each mitotic figure, observations were made of the mitotic figures in the growing glumes of very young florets in those plants that showed very few recovered sectors (that is, nonvariegated sectors which arise following healing of the broken end in the precursor cell or cells that gave rise to the sector). Since healing of the broken end was rare in these plants, many of the mitotic figures in these glumes could be expected to show a bridge configuration at anaphase. Counts were confined to mid and late anaphases and to the very early telophase figures. Accurate observations of bridge configurations could not be made

in the earlier anaphases because of the crowding of the chromosomes in the spindle figure. The results are given in the following table:

Mid to late anaphase:	
Single bridge	234
No bridge	77
Early telophase:	
Single bridge	92
No bridge	299

In many cases where a scoring of "no bridge" was made, it was obvious that a bridge had been present but had been broken by tension shortly before fixation had occurred. This applies particularly to the late-anaphase figures, although a number of such figures were observed at early telophase. Breakage of a chromatin bridge may occur at relatively early anaphase. However, breakage of the bridge may not occur at either the anaphase or the following telophase, for a fine chromatin bridge connecting two resting nuclei was frequently observed. The time of breakage of a bridge configuration may be related, in part, to the absolute length of the chromatin in the bridge. Among the different cells, this may vary from very short to very long. The time of breakage may likewise be governed, in part, by the length of the cell itself, which controls the extent to which the spindle may elongate. When the spindle axis is long, a short bridge may be broken early. When the axis is compressed, even a short bridge may not be broken in this spindle figure.

That it was the broken chromosome 9 contributed by the male parent which continued the chromatid type of breakage-fusion-bridge cycle, from the time of the first breakage at meiosis in the male parent through the gametophytic divisions and then through the consecutive sporophytic divisions, was shown by examination of pachytene configurations in these plants. When the variegation continued into the microsporocyte tissues, the recognizable deficient chromosome 9 contributed by the female parent and a newly broken chromosome 9 contributed by the male parent were evident. The chromatin constitution of the short arm of the newly broken chromosome 9 varied, however, from cell to cell within the same anther. Various types of duplication, reduplication, deficiency, and deficiency plus duplication of the short arm were recognizable among the many microsporocytes examined. In these cells, the two sister chromatids of the newly broken chromosome 9 were fused at the position of the previous breakage, which occurred in the last premeiotic anaphase. This was evident from a study of the meiotic anaphases in those anthers where the breakage-fusion-bridge cycle had continued into meiosis. A single chromatid bridge *with no accompanying fragment* was present in many sporocytes, in either the first or the second meiotic anaphase. It could appear in the first meiotic mitosis if a crossover had occurred between one of the fused chromatids and a chromatid of the mitotically normal homologue. In the anthers examined, it most frequently appeared in the second meiotic anaphase, as shown by the table:

First meiotic anaphase:	
Single bridge	20
No bridge	64
Second meiotic anaphase:	
Single bridge in one cell of dyad	83
No bridge in either cell of dyad	25

In the second division anaphases, the dyads showing no bridge configuration usually gave evidence that a bridge had occurred in the first meiotic mitosis (for type of evidence, see McClintock, 1938). It may be concluded, then, that each sporocyte will show a chromatid bridge at a meiotic anaphase if it has received a chro-

mosome which was broken at the last premeiotic anaphase, provided that the broken end has not healed in the interim.

These studies of bridge configurations in somatic and meiotic mitoses strongly support the conclusion that a single chromatin bridge configuration will occur in every successive mitosis until the broken end is healed by some, as yet undetermined, cause. This healing may occur at any time. If the broken end had healed in one or more of the archesporial cells, clusters of sporocytes within an anther should show the same chromatin constitution of the short arm of the broken chromosome 9. This was observed in the pachytene examinations of a few of the anthers of the highly variegated plants.

It is now realized that it is possible for the chromatid type of breakage-fusion-bridge cycle to continue indefinitely. Most of the plants with chromatid bridges, however, appeared in only 4 of the 188 cultures. This suggests that some controlling genetic factors may be responsible for the continuation of the chromatid bridge cycle into the sporophytic tissues.

Homozygous Deficiency as a Cause of Mutation in Maize

Methods of obtaining internal minute deficiencies. It has been demonstrated that the recessive mutations *pyd* and *wd,* which are comparable in genetic behavior to typical recessive mutants, arise following deletions of small terminal segments of the short arm of chromosome 9. It has likewise been shown that deletions of small segments of chromatin in a region adjacent to the centromere of the short arm of chromosome 5 will give rise to visible mutations. A number of such mutants, which were repeatedly produced, have been identified. One of these exactly simulated and was allelic to the previously known recessive mutant *bm*-1 (brown midrib; see McClintock, 1941) and appeared when the *Bm*-1 locus had been removed from the chromosome; that is, when the plant was homozygous deficient for the *Bm*-1 locus. Selection of these two regions of the genomic complement for studies of homozygous deficiencies was purely a matter of chance and was governed by structural abnormalities which happened to occur in these two regions. It is reasonable to believe, therefore, that many mutations, distributed throughout the whole chromosome complement in maize, could arise as the consequence of homozygous minute deficiencies. Studies are now under way to test this hypothesis by subjecting the short arm of chromosome 9 to processes which could produce minute internal deficiencies within this arm of the chromosome. Several methods are being employed. One method utilizes the chromatid bridge cycle, which sometimes brings about the deletion of small internal segments of the short arm of chromosome 9. It has been observed that the tension on a chromatin bridge at a mitotic anaphase occasionally may result in breakages of this bridge at more than one position between the two centromeres. The fragments produced may or may not enter one of the telophase nuclei. Following this type of breakage, and following particular types of fusion of the broken ends in the succeeding nucleus, it is possible to obtain a chromosome 9 having an internal deficiency within the short arm. An internal deficiency may likewise be produced if the anaphase chromosomes are composed of two sister strands. When a bridge configuration is present, breakage of the two strands at anaphase may occur at a different position within each strand. If this is followed in the succeeding nucleus by fusion of the broken ends of the two unequal sister strands, and if a particular

position of breakage occurs in the bridge at the next anaphase, an internal deficiency may arise in one of the broken chromosomes. Should the broken end heal, a chromosome 9 with an internal deficiency could be isolated.

Since it is known that the chromatid type of breakage-fusion-bridge cycle may bring about internal deficiencies, mutations other than *pyd* and *wd* could appear in some of the seedlings which were grown to test the frequency of appearance of *pyd* and *wd* (as described in the second section of this report). In the cross that gave rise to these seedlings, the female parent contributed a chromosome 9 deficient for nearly one-third of the terminal segment of the short arm. The male parent contributed a chromosome 9 which had undergone the breakage-fusion-bridge cycle in at least the preceding gametophytic divisions. If this chromosome has an internal deficiency which lies within the range of the deficiency in the chromosome 9 contributed by the female parent, a seedling with a visible mutation could appear, provided that the homozygous deficiency allows viable tissues to be formed. An occasional seedling with an obvious mutation other than *pyd* and *wd* did appear among the 3287 seedlings. Some were viable and these mutants are now being tested to determine whether they are located within the short arm of chromosome 9 and whether they are associated with a visible structural modification of the arm. Other types, particularly those with defective chlorophyll, were inviable; but the isolation of such mutants for further study is being conducted by a method which is more laborious but which will insure their genetic perpetuation.

A very striking confirmation of the production of mutations other than *pyd* and *wd* by the chromatid type of breakage-fusion-bridge cycle was observed in the plants that have a deficient chromosome 9 contributed by the female parent and a newly broken chromosome 9 contributed by the male parent, this newly broken chromosome 9 having *continued* the breakage-fusion-bridge cycle into the sporophytic tissues (see the third section of this report). In these plants, sectors of mutant tissue appeared. Such sectors are to be expected following structural modifications within the short arm of chromosome 9 which arise during the successive chromatid bridge cycles. Should a deletion of a small segment occur within the range of the deficiency in the female chromosome 9, and should this broken chromosome subsequently heal, a sector of homozygous deficient tissue could be initiated. As might be expected, many pale-yellow and white sectors were observed. Also, in some cases, the phenotypic characters of these sectors were similar to those observed in the new mutant seedlings mentioned above. Mutant sectors of the same phenotype were observed in different plants. This is to be expected on the basis of the hypothesis outlined, for the same mutant should appear whenever the same homozygous deficiency is present.

A second method of obtaining internal deficiencies in the short arm of chromosome 9 is based on the behavior of dicentric chromosomes 9. The dicentric chromosome is formed following the fusion of a broken end of the short arm of the chromosome 9 introduced by the female gamete with a similar chromosome 9 introduced by the male gamete. The behavior of this dicentric chromosome has been described in the two previous reports. Following a succession of the *chromosome* type of breakage-fusion-bridge cycle, the two broken ends entering a nucleus may heal and the two chromosomes 9 may be mitotically normal from then on. While this chromosome type

of breakage cycle is in progress, segments of the short arm are continually shifting their positions. This may result in the production of an internal deficiency in one or both of the healed broken chromosomes 9. If these healed broken chromosomes with internal deficiencies are male and female transmissible, it should be possible to obtain plants homozygous for these internal deficiencies. Mutations due to these deficiencies could then appear. New mutants other than *pyd* and *wd* have been isolated from the progeny of these plants. One of these mutants is phenotypically similar and genetically allelic to one of the mutants obtained by the chromatid type of bridge cycle outlined above.

The repeated occurrence of the yg-2 phenotype following chromosome breakage. Other than *pyd* and *wd,* the most frequently recognized mutant arising from the chromosome and chromatid bridge cycles simulates in detail the phenotypic expression of the known recessive mutant *yg-2* (yellow-green plants). This mutant is located in the terminal chromomere of the short arm of chromosome 9. As stated previously, 77 *pyd* and 48 *wd* mutants appeared in the progeny of the 3287 seedlings derived from zygotes that had received a deficient chromosome from the female parent and a newly broken chromosome 9 from the male parent. There were, in addition, 6 yellow-green mutants. Four of these died following transplantation from the seedling bed, but 2 survived. Cytological examination of the chromosomes 9 in these 2 surviving plants showed that the male parent had introduced a newly broken chromosome 9 and that the female parent had contributed the chromosome 9 with the long terminal deficiency. No terminal deficiency was present, however, in the newly broken chromosome 9. When each of these two newly broken chromosomes was combined with a chromosome carrying the normal *yg-2* mutant, yellow-green plants appeared. Thus, these two newly derived yellow-green mutants are allelic to *yg-2*. When combined with *wd,* both of the new yellow-green mutants gave yellow-green plants; in this respect, they are similar to *yg-2*. They also gave yellow-green plants when combined with each other. A third yellow-green mutant, derived from a dicentric chromosome 9 which had undergone the chromosome bridge cycle, behaved similarly in its phenotypic and genetic expressions. Furthermore, the typical yellow-green phenotype appeared as a recovered sector in plants starting development with a dicentric chromosome 9 and in plants with a deficient chromosome 9 and a chromosome 9 undergoing the chromatid type of bridge cycle. In several cases, these sectors extended into the sporogenous tissues, allowing the broken chromosome carrying the new yellow-green mutation to be isolated. At present, 7 new yellow-green mutants are being tested.

The relatively frequent and independent occurrence of mutants having the yellow-green phenotype suggests that they may be caused by a homozygous small internal deficiency. It is realized that many of the internal deficiencies which are male and female transmissible and which produce visible mutations when homozygous may not be cytologically recognizable. This is because the piece deleted may be too short for an accurate determination. Such deficiencies, however, *must* be produced by the chromosome and chromatid types of breakage-fusion-bridge cycles. Because of our accumulating knowledge that homozygous minute deficiencies will give rise to mutant phenotypes, it is expected that some of these deficiencies will produce visible mutations. Furthermore, the methods should produce the same mutation

time and again. This has been shown for *pyd* and *wd,* where the deficiencies are cytologically obvious, and for yellow-green mutants, where the deficiency may not be so easily detected. An intensive study of the chromosomes carrying these new yellow-green mutations will be conducted.

Preliminary evidence suggesting that the bz phenotype may be simulated by a homozygous deficiency of the Bz locus. The recessive mutant *bz* (bronze) described by Rhoades produces a "bronzing" of both aleurone and plant color. In the plant cells, the bronze character is due to the presence of brown pigment in addition to red or purple pigment. Only a small amount of purple or red pigment develops in the aleurone grains of endosperms that are homozygous *bz*. This mutant is located in the short arm of chromosome 9 between the mutant *C* and the centromere (Rhoades, unpublished). A chromosome 9 that had undergone the chromatid bridge cycle for several nuclear generations and is deficient for a total of approximately $1\frac{1}{2}$ chromomeres has been isolated. Two deficiencies are present in this chromosome: a small terminal deficiency, which gives rise to *pyd* when homozygous, and a longer internal deficiency located between *C* and the centromere. When this deficient chromosome was combined with a normal chromosome carrying *bz,* the bronze phenotype appeared in both the aleurone and the plant. This suggested that the *Bz* locus is included in the internal deficiency. The tests that have been conducted so far have shown that the bronze phenotype will appear when the aleurone and plant tissues are homozygous deficient for the internal deficiency of this chromosome. When this deficient chromosome, minus the *Bz* locus, is introduced into the primary endosperm nucleus by the female parent, and a newly broken chromosome 9 with *Bz* is introduced by the male parent, an endosperm develops which is variegated for sectors of homozygous deficient tissues. This is due to the chromatid type of breakage-fusion-bridge cycle which the chromosome introduced by the male parent undergoes. This cycle constantly deletes segments from the short arm of this chromosome during the development of the endosperm. The sectors that are homozygous deficient for the terminal deficiency are completely normal in appearance, but the sectors that are homozygous deficient for the internal deficiency are similar in phenotypic appearance to homozygous *bz* if not indistinguishable from it. As yet, only one plant has been available to test the effect of the homozygous deficiency on plant color. This plant was variegated for normal and homozygous deficient tissues. In the limited regions where plant color developed, sectors of normal color (*Bz*) and sectors that were similar to the recessive *bz* phenotype were observed. The colored cells in normal (*Bz*) plants and in bronze (*bz bz*) plants were compared microscopically with the normal and the bronzelike sectors of the variegated plant. As far as these observations permit a judgment, the type of color modification in the homozygous deficient sectors of the variegated plant was similar to that in homozygous *bz* plants. Although the evidence is preliminary, it strongly suggests that the bronze phenotype may be simulated by a homozygous deficiency of the *Bz* locus. It is possible that the recessive mutant *bz,* or a deficiency of the *Bz* locus, exerts its effect on the interrelated anthocyanin-anthoxanthin synthesis in the cell. The investigations are being conducted in cooperation with Dr. M. M. Rhoades.

This deficient chromosome is transmitted normally through the female gametophyte. It is worthy of note that this chromosome with a relatively long total deficiency is partially male transmissible. Plants pos-

sessing one normal chromosome 9 and this deficient chromosome 9 produce pollen one-half of which carries the normal chromosome and one-half of which carries the deficient chromosome. When this pollen was placed on silks of normal plants, 1092 of the 9689 functional pollen grains tested carried the deficient chromosome.